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Effect of Acute Administration of Celecoxib on Anxiolytic Activity of Fluoxetine in Albino Mice

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ABSTRACT

Depression and anxiety often co-exist and Selective Serotonin reuptake inhibitors (SSRIs) like fluoxetine are widely used clinically for treatment of depression, especially with concomitant anxiety. Patients on fluoxetine may be required to take selective COX2 inhibitors like celecoxib for any inflammatory or painful conditions. Our objective was to evaluate the effect of acute administration of celecoxib on the anxiolytic activity of fluoxetine. After clearance from Institutional Animal Ethics Committee, 30 healthy albino mice (20 - 30g) of either sex were divided into five groups of six mice each and administered single dose of Distilled water 1ml/kg (control), Alprazolam 5mg/kg (standard), Fluoxetine 5mg/kg, Fluoxetine 5mg/kg + Celecoxib 5mg/kg and Alprazolam 5mg/kg + Celecoxib 5mg/kg respectively by intraperitoneal route. Testing for anxiolytic effect was done by Elevated Plus Maze (EPM) and Hole Board (HB) test in all groups at baseline (Day 0) and Day 1 after drug administration. Open arm exploration time in EPM and number of times of nose pokings in HB were measured in all groups and the results were expressed as Mean ± SD. Statistical analysis was done by one-way ANOVA followed by Unpaired 't' test with $P<0.05$ as the level of significance (95% confidence limits). Open arm exploration time and number of nose pokings were significantly decreased after drug administration in fluoxetine + celecoxib group in EPM and HB, compared to normal control. Thus, we observed contrasting results (anxiogenic and anxiolytic activity in EPM and HB respectively) when we co-administered fluoxetine and celecoxib.

Keywords: Anxiolytic, Fluoxetine, Celecoxib, Elevated Plus maze, Hole Board

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INTRODUCTION

Anxiety is an unpleasant emotional state associated with uneasiness, worry, tension and concern for the future. Depression and anxiety often co-exist, as in Mixed Anxiety and Depressive Disorder (ICD 10) [1]. Selective Serotonin reuptake inhibitors (SSRIs) like fluoxetine are widely used clinically for treatment of depression, but they are also administered for treatment of anxiety and panic disorder [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) which are used to treat inflammatory and painful conditions may be non-selective cyclo-oxygenase (COX) or selective COX-2 inhibitors. Selective COX-2 inhibitors like celecoxib are approved for management of acute pain in adults, treatment of osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis and primary dysmenorrhoea [3]. A major adverse effect of NSAIDs, especially non-selective COX-inhibitors, is the increased risk of gastric ulcers and gastrointestinal bleeding, which occur less with selective COX-2 inhibitors.

Patients on fluoxetine for anxiety or Mixed Anxiety and Depressive Disorder may be required to take NSAIDs for any inflammatory or painful conditions. Even though selective COX-2 inhibitors are not first choice NSAIDs due to their adverse cardiovascular profile, there may be a case for their judicious use in such patients based on their gastrointestinal tolerability. This is because fluoxetine and other SSRIs cause acid peptic disease and a meta-analysis has proved that there is an increased risk of gastrointestinal bleeding with the concurrent use of NSAIDs and SSRIs [4]. Hence, celecoxib may be a viable alternative in those conditions.

Effect of administration of NSAIDs on the antidepressant activity of fluoxetine has been studied earlier [5-7]. But there is a lack of scientific literature on the effect of such concurrent administration on the anxiolytic effect of fluoxetine. Hence, we wanted to observe the acute effect of administration of celecoxib on the anxiolytic activity of fluoxetine in animal models of anxiety.

Our objective was to evaluate the anxiolytic activity of fluoxetine on concurrent administration of celecoxib in albino mice.

MATERIALS AND METHODS

We performed the following experiments after obtaining clearance from the Institutional Animal Ethics Committee.

Experimental Animals

We obtained thirty healthy adult albino mice of either sex (20-40g) from the Central Animal House of our institute and kept them for one week in the departmental animal house, grouped in separate cages. We ensured maintenance of 12 hours light:dark cycle and free access to laboratory diet and water, as per the recommendations of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) [8].

Drugs and Doses

We acquired the following drugs – fluoxetine, ibuprofen and celecoxib from Cadila Pharmaceuticals Ltd., J&K, Abbott India Ltd., Goa and Zydus Cadila, Zydus Healthcare, Sikkim respectively. We selected low doses of fluoxetine and celecoxib and standard dose of alprazolam from previous studies [9-11]. We suspended the drugs in Distilled water (D/W) (1ml/kg) and administered Fluoxetine 5mg/kg, Alprazolam 5mg/kg (standard anxiolytic) and Celecoxib 5mg/kg intra-peritoneally.

Grouping and Treatment Scheduling

We divided healthy albino mice of either sex (20-40g) into five arms containing six mice each for testing antidepressant activity (n=30). The treatment schedule was as follows:

Group A: D/W (1ml/kg)
Group B: Alprazolam (5mg/kg)
Group C: Fluoxetine (5mg/kg)
Group D: Fluoxetine (5mg/kg) + Celecoxib (5mg/kg) administered separately
Group E: Alprazolam (5mg/kg) + Celecoxib (5mg/kg) administered separately

Experimental Design

We performed the test for anxiolytic effect by Elevated plus maze (EPM) and Hole Board (HB). After taking baseline values of tests with EPM and HB on Day 0, the vehicle and the drugs were administered orally 30 minutes before subjecting them to EPM and HB tests on Day 1.

Elevated Plus Maze (EPM)

The elevated plus maze test, first described in 1958[12-14], has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time whereas anxiogenic compounds have the opposite effect [15-19].

The plus-maze consisted of two open arms, 115 × 10 cm (L ×W), and two enclosed arms, 40 × 50 × 12cm (L ×W×H), open upwards, arranged so that the two open arms were opposite to each other. The maze had an elevation of 50 cm from the floor. Thirty minutes after intraperitoneal administration of the test/standard drug, we placed the mice in the centre of the maze, facing one of the enclosed arms. During a 5min testing period, we calculated the time spent in the open and enclosed arms using a stop watch.

Open arm exploratory time was noted. The values of treated groups were compared with controls.

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Hole Board Test

The evaluation of curiosity or exploratory behaviour of mice was first reported by Boissier, *et al* [19]. In this test, we used an open field with holes on the bottom into which the animals could poke their noses. The hole-board had a size of 40 × 40 cm. Sixteen holes with a diameter of 4 cm each were distributed evenly on the floor. The board was elevated to a height of 32 cm, so that the mouse poking its nose into the hole does not see the bottom. Nose-poking is a typical behaviour of mice indicating a certain degree of curiosity and is measured by visual observation. Anxiolytic drugs suppress nose poking behaviour whereas anxiogenic drugs increase such activity. Thirty minutes after intraperitoneal administration of the test/standard compound, the first animal was placed on the hole-board. We observed the number of times the mouse poked its nose into the hole during the 5minute testing session.

The number of counts for nose-poking of treated animals was compared with those of control.

Statistical Analysis

We performed Statistical analysis, using SPSS statistical software Version 16.0. Duration of immobility was expressed as Mean ± SD. For demonstration of anxiolytic activity, we used one way ANOVA, followed by Unpaired 't' test for analysing the difference between groups (if any), with $P < 0.05$ as level of significance with 95% confidence interval.

RESULTS

The results of EPM and HB expressed as Mean ± SD are shown in Table 1 and results of one way ANOVA are shown in Table 2. Inter-group comparisons on day of experiment (Day 1) using Unpaired 't' test are shown in Tables 3 and 4.

Table 1: Cumulative duration of time (seconds) spent in the open arm in Elevated Plus Maze (EPM) and Number of times of nose poking in Hole Board (HB), expressed as Mean ± SD

GROUP	EPM (D0)	EPM (D1)	HB (D0)	HB (D1)
A	21.17 ± 11.89	13.67 ± 5.35	34.83 ± 7.00	34.83 ± 6.05
B	16.50 ± 5.96	49.67 ± 14.57	40.33 ± 13.05	20.83 ± 9.02
C	17.50 ± 6.97	34.67 ± 13.89	31.83 ± 5.38	28.83 ± 4.36
D	17.50 ± 8.26	5.83 ± 3.87	50.00 ± 10.28	1.83 ± 1.83
E	16.33 ± 5.24	131.67 ± 41.08	38.17 ± 3.66	18.33 ± 3.39

Table 2: Results of One Way ANOVA

Test	Day 0 (*P* value)	Day 1 (*P* value)
EPM	0.78	0.00**
HB	0.07	0.00**

P value < 0.05, not significant on Day 0, highly significant (**) on Day 1

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Table 3: Results of Unpaired 't' test between groups on day of experiment (Day1) for EPM

t-test		A	B	C	D	E
A	t=					
	p=					
B	t=	5.68				
	p=	0.00**				
C	t=	3.45	1.83			
	p=	0.01**	0.10			
D	t=	2.91	7.12	4.90		
	p=	0.02*	0.00**	0.00**		
E	t=	6.98	4.61	5.48	7.47	
	p=	0.00**	0.00**	0.00**	0.00**	

*indicates significant and ** indicates highly significant difference between groups

Table 4: Results of Unpaired 't' test between groups on day of experiment (Day1) for HB

t-test		A	B	C	D	E
A	t=					
	p=					
B	t=	3.16				
	p=	0.01**				
C	t=	1.98	1.96			
	p=	0.08	0.08			
D	t=	12.79	5.06	13.10		
	p=	0.00**	0.00**	0.00**		
E	t=	5.83	0.63	4.66	10.49	
	p=	0.00**	0.54	0.00**	0.00**	

*indicates significant and ** indicates highly significant difference between groups

DISCUSSION

Our objective was to evaluate the effect of acute administration of celecoxib on the anxiolytic activity of fluoxetine in albino mice. We found that in alprazolam, fluoxetine and alprazolam combination with celecoxib groups, there was a mean increase in the time spent in the open arm in EPM. Thus, these groups demonstrate anxiolytic activity. However, in the control and combination group of fluoxetine with celecoxib, we observed a mean decrease in the time spent in the open arm, demonstrating a lack of anxiolytic activity compared to the control group. In HB test, mean number of nose pokings decreased in all groups compared to the control, demonstrating anxiolytic activity.

Results of one way ANOVA showed that there was no significant difference between the performances of the groups in EPM and HB test on Day 0. Thus, the groups were comparable at baseline before drug administration. But, we observed a highly significant difference between the performances of the animals in EPM and HB test on Day 1 (after drug administration) compared to Day 0.

On comparing the performance of the drug treated groups in EPM on Day 1, alprazolam, fluoxetine and alprazolam with celecoxib groups showed significant anxiolytic activity compared to control. However, there was a significant decrease in anxiolytic activity in the combination group of fluoxetine and celecoxib compared to control as well as all other groups. Acute administration of celecoxib with fluoxetine probably reversed the significant anxiolytic activity which was seen after administration of fluoxetine alone.

On comparing the performance of the drug treated groups in HB on Day 1, alprazolam and combination groups of fluoxetine and alprazolam with celecoxib showed significant anxiolytic activity compared to control. However, there was no significant anxiolytic activity with fluoxetine alone, compared to control. The combination group of fluoxetine and celecoxib demonstrated significant anxiolytic activity compared to all groups. Concluding from the results of this test, acute administration of celecoxib with fluoxetine probably imparted significant anxiolytic activity, which was not seen when fluoxetine was administered alone.

Thus, we observed opposing results (anxiolytic as well as anxiogenic) from the two tests when we evaluated the effect of single dose acute administration of celecoxib on the anxiolytic effect of fluoxetine in adult albino mice.

SSRIs were initially introduced in therapeutics as antidepressants and their potential as anxiolytics has been observed in the treatment of social phobia, post-traumatic stress disorder, and generalized anxiety disorder. In humans, the anxiolytic effects of SSRIs emerged only after chronic treatment, and there are some reports that they may initially increase anxiety [20].

In preclinical studies, the acute effects of SSRIs were detected in several procedures that are used for characterizing anxiolytic drugs, but the nature of these effects varies markedly with the experimental procedure [21,22]. Some studies found no effect of SSRIs, whereas others showed decrease or increase in anxiety-like behaviour. A viable hypothesis for these SSRI effects depends on whether the experimental procedure measures conditioned or unconditioned behaviours, particularly when the SSRI is given acutely [21,23]. Consistent anxiolytic-like effects of SSRIs were observed on measures of various types of behaviours [24-30]. Anxiogenic-like effects of SSRIs have been observed on light dark exploratory behaviour[24] and the elevated plus maze [31]. The anxiogenic effect seen after acute fluoxetine administration could be related to the increased extracellular 5-HT around subcortical structures, observed after acute administration of SSRIs [32].

However, previous literature on the effect of NSAIDs on anxiolytic activity of SSRIs is lacking, so we were not able to compare our study with similar studies.

CONCLUSION

Our objective was to evaluate the effect of acute administration of celecoxib on the anxiolytic activity of fluoxetine, using elevated plus maze and hole board tests in albino mice. We observed that concurrent administration of celecoxib decreased the anxiolytic activity of fluoxetine in elevated plus maze. We also found that anxiogenic effect of

fluoxetine alone was reversed by the combination with celecoxib in hole board test. From these observations, it is difficult to interpret whether the combination has increased or decreased the anxiolytic activity of fluoxetine. Anxiogenic effect of fluoxetine was also observed in some earlier acute studies. Even in humans, the same phenomenon can be observed and consistent anxiolytic effects are seen with chronic administration of SSRIs only. Therefore, we have to continue the study for a longer duration in order to understand the effect of administration of celecoxib on the anxiolytic activity of fluoxetine. This will help us to detect whether there is a drug interaction leading to decrease or increase in anxiolytic activity when celecoxib is taken by any patient on SSRIs, such as fluoxetine.

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